Transition on the entropic elasticity of DNA induced by intercalating molecules

M. S. Rocha, M. C. Ferreira, and O. N. Mesquita

Departamento de Física, ICEX, Universidade Federal de Minas Gerais,

Caixa Postal 702, Belo Horizonte, CEP 31270-901, MG, Brazil

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Abstract

We use optical tweezers to perform stretching experiments on DNA molecules when interacting with the drugs daunomycin and ethidium bromide, which intercalate the DNA molecule. These experiments are performed in the low-force regime from zero up to 2 pN. Our results show that the persistence length of the DNA-drug complexes increases strongly as the drug concentration increases up to some critical value. Above this critical value, the persistence length decreases abruptly and remains practically constant for larger drug concentrations. The contour length of the molecules increases monotonically and saturates as drugs concentration increases. Measured intercalants critical concentrations for the persistence length transition coincide with reported values for the helix-coil transition of DNA-drug complexes, obtained from sedimentation experiments.

Key words: DNA; daunomycin; ethidium bromide; persistence length; optical tweezers; single molecule

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I. INTRODUCTION

DNA-drug interactions have been much studied along the past years. An important motivation for these studies is the fact that many of the studied drugs are used for treatment of human diseases, particularly, in cancer chemotherapy.

Single molecule stretching experiments using optical tweezers have yielded a great amount of information about DNA-protein and DNA-drug interactions [1, 2, 3, 4, 5, 6].

Recently, we studied the interaction between psoralen and DNA when illuminated with ultraviolet light A (UVA). Psoralen is a drug used to treat some skin diseases, like psoriasis and vitiligo. This drug intercalates the DNA molecule and can form covalent linkages with the thymines if the complex is illuminated with ultraviolet light, modifying drastically its elasticity and impeding the DNA replication and transcription. The persistence length of the DNA-psoralen complexes formed after UVA illumination were measured in [7].

Daunomycin and ethidium bromide (EtBr) are other examples of drugs which intercalate the DNA molecule and can modify its elasticity, depending on the drug concentration. Both drugs unwind the DNA double helix when intercalating [8]. Daunomycin is an anthracycline antibiotic used in the treatment of various cancers. It inhibits DNA replication and transcription when intercalating, impeding cell duplication [9]. Ethidium bromide (EtBr) is commonly used as a non-radioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and in other methods of nucleic acid separation.

Several works have reported different results for the effects of these drugs on the entropic elasticity of DNA molecules. In those works, the measured parameter used to study elasticity modifications is the persistence length of the DNA-drug complex. Smith *et al.* [2] report that ethidium bromide does not modify the elasticity of the DNA molecule, only increasing its contour length by $\sim$40%. Tessmer *et al.* [10] report that ethidium bromide causes a large increase in the contour length and a decrease in the persistence length of the complex for 1 μM of the drug, and at lower concentrations, an increase in both persistence and contour lengths. Recently, Sischka *et al.* [11] report the value of 28.1 nm for the persistence length of DNA-daunomycin complexes and 20.7 nm for DNA-EtBr complexes, smaller than the bare DNA persistence length of about 50 nm. The authors have used in this work a concentration of 1 μM for both drugs, and a DNA concentration of 15 pM. In the present work, in order to clearly establish the effect of these intercalating drugs on the persistence length of the DNA

complexes, we performed stretching experiments at various drugs concentrations, from zero up to saturation of the complexes. We show that the values obtained for the persistence length depend strongly on the concentration ratio between drug and DNA base pairs. Our results show that the persistence length of the complexes increases as we increase the drug concentration until certain critical concentration is reached. Above this critical concentration the persistence length decreases abruptly and remains practically constant for larger drug concentrations.

II. EXPERIMENTAL PROCEDURE

To measure the persistence and contour length of DNA molecules and DNA-drug complexes, we use optical tweezers [1, 2, 3, 4, 5, 6] and intensity autocorrelation spectroscopy [12].

The samples consist of λ-DNA molecules in a PBS pH 7.4 with [NaCl] = 140 mM solution. We attach one end of the molecule to a microscope coverslip, and the other end is attached to a polystyrene bead. To do this, we use the procedure described in [13]. We add the drug in the sample immediately before the measurements. The DNA concentration used in all experiments was $C_{DNA} = 6.81$ μg/mL, which corresponds to a base pairs concentration of $C_{bp} = 11$ μM.

Our optical tweezers is mounted in a Nikon TE300 microscope with an infinite corrected objective (100X, N.A. = 1.4). The trapping laser is an infrared (IR) laser with $\lambda = 832$ nm (SDL, 5422-H1). The optical tweezers is used to trap the polystyrene bead attached to the end of the DNA molecule, so we can manipulate and stretch the DNA molecule.

In addition, we use a He-Ne laser ($\lambda = 632.8$ nm) as the scattering probe. The backscattered light by the polystyrene bead is collected by a photodetector, which delivers pulses to a digital correlator. We, then, obtain the autocorrelation function of the backscattered light, which allows us to determine the stiffness of the optical trap, due to the Brownian motion of the trapped bead.

The next step in the experimental procedure is to obtain the force *versus* extension curves for the DNA molecules and DNA-drug complexes. To do this, we use the optical tweezers to trap the bead with the DNA while pulling the microscope slide, stretching the DNA. The backscattered light is collected while stretching the DNA. From the backscattered light

intensity one obtains the displacement of the trapped bead in relation to its equilibrium position, and by multiplying it times the tweezers' stiffness, the force exerted by the DNA molecule while it is stretched is obtained. The details about our experimental setup and experimental procedure can be found in [7, 12].

Finally, with the force *versus* extension curves, we use the approximate expression derived by Marko and Siggia (Eq. 1) [14] to obtain the persistence and contour length of the DNA molecules and DNA-drug complexes,

$$F = \frac{k_B T}{A}\left[\frac{z}{L} + \frac{1}{4\left(1-\frac{z}{L}\right)^2} - \frac{1}{4}\right], \tag{1}$$

where z is the DNA molecule end-to-end distance, k_B is the Boltzmann constant, T is the absolute temperature, A is the DNA persistence length and L is the DNA contour length.

Figure 1 is a typical force *versus* extension curve obtained with this procedure, for a drug-free DNA molecule. Fitting this curve with Eq. 1, we extract the persistence length ($A = 50 \pm 3$ nm) and the contour length ($L = 16.5 \pm 1$ μm) for the λ-DNA. These values correspond to well-known values reported in the literature [3, 15, 16].

III. RESULTS AND DISCUSSION

In this section we show the results obtained for the two drugs used: daunomycin and ethidium bromide.

A. Daunomycin

We have performed experiments with DNA-daunomycin complexes for several drug concentrations. In Fig. 2 we show the persistence length (A) of the complexes as a function of total daunomycin concentration (C_D) for fixed DNA base pairs concentration of $C_{bp} = 11$ μM. We denote by C_D the total daunomycin concentration used to prepare the sample, which is the sum of both the bounded to DNA and the free drug concentration in solution.

The point which the drug concentration is zero corresponds to the drug-free DNA situation with $A = 50$ nm.

The behavior of the persistence length A as a function of daunomycin concentration C_D can be described as follows: it initially increases with C_D until it reaches a maximum value

($\sim$ 280 nm) at the critical concentration $C_D^{critical} = 18.3$ μM. Then, it decays abruptly to around 75 nm and remains practically constant at this value even if we continue to increase C_D. The contour length increases monotonically from 16.5 $\pm$ 1 μm up to the saturation value of 21 $\pm$ 1 μm. These mean values are obtained performing an average over many different DNA molecules and DNA-drug complexes. Such distribution of contour length values was also observed by Mihailovic *et al.* [17].

Also, we can estimate the exclusion parameter n (number of total base pairs divided by the number of total intercalated drug molecules) from our experimental data. The average value of the contour length for DNA-daunomycin complexes obtained when using a saturated concentration of the drug increases about 27% relative to drug-free DNA contour length (16.5 μm). This means that when all possible drug molecules are intercalated, the DNA increases its contour length by 4.5 μm. Knowing that each intercalated daunomycin molecule increases the contour length of the complex by 0.31 nm [8], we determine the total number of intercalated daunomycin molecules, which is around 14500. Finally, the exclusion parameter can be obtained by dividing the number of base pairs of the λ-DNA (48500) by the number of total intercalated drug molecules (14500). We obtain $n = 3.3$, in good agreement with the value 3.5 reported in [9].

For comparison purposes, Fig. 3 shows two force *versus* extension curves (normalized by the contour length) for two daunomycin concentrations, before and after the transition. The data points in this figure are smoothed for better visualization, *i. e.*, Brownian fluctuations are averaged out.

B. Ethidium Bromide (EtBr)

The behavior of the persistence length as a function of the drug concentration for DNA-EtBr complexes is very similar to the DNA-daunomycin complexes. The difference is that in this case the transition occurs at a lower drug concentration (see Fig. 4) for the same DNA base pairs concentration $C_{bp} = 11$ μM. The maximum value measured for the persistence length of DNA-EtBr complexes is $\sim$ 150 nm, at the critical concentration $C_E^{critical} = 3.1$ μM. The contour length increases monotonically from 16.5 $\pm$ 1 μm up to the saturation value of 23 $\pm$ 1 μm. Again, these values for the contour lengths are averages over many different molecules.

Repeating the same calculation for the exclusion parameter of EtBr, which increases the DNA contour length by 0.34 nm per intercalated molecule [11], we obtain $n = 2.5$, in reasonable agreement with the value 2.01 reported in [18].

C. Equilibrium binding constants

In our experiments we control the total drug concentration C_T and the total concentration of DNA base pairs C_{bp}. To discuss the elastic properties of the DNA complex formed the important parameter to consider is the ratio r between the concentration of bounded drug (C_b) per concentration of DNA base pairs (C_{bp}). In order to obtain r, the binding of molecules to DNA is analyzed using the neighbor exclusion model [9]. A closed form for this model was obtained by McGhee and von Hippel [19] and can be expressed by the equation

$$\frac{r}{C_f} = K_i(1-nr)\left[\frac{1-nr}{1-(n-1)r}\right]^{n-1}, \tag{2}$$

where r is ratio between the concentration of bounded drug (C_b) per concentration of DNA base pairs (C_{bp}), C_f is the concentration of free drug (not bounded), K_i is the intrinsic binding constant and n is the exclusion parameter in base pairs. For a more detailed discussion about the neighbor exclusion model, see [19].

The concentration of free drug (C_f) can be simply related with the concentration of bounded drug (C_b) and the total drug concentration (C_T) through the equation

$$C_T = C_f + C_b. \tag{3}$$

Using Eq. 2 and 3 with the determined exclusion parameter ($n = 3.3$), the intrinsic binding constant reported in [9] for daunomycin, $K_i = 7\times10^5$ M^{-1}, the critical daunomycin concentration measured in this work ($C_D^{critical} = 18.3$ μM), and the concentration of DNA base pair used in our experiments ($C_{bp} = 11$ μM), we can determine the critical ratio r_c, which we define as the value of r at the abrupt transition for the value of the persistence length. We then obtain the value $r_c = 0.248$.

Similarly, for EtBr, we use the parameters $n = 2.5$, $K_i = 1.5\times10^5$ M^{-1} [20], and $C_E^{critical}$ $= 3.1$ μM determined again from the abrupt change in persistence length. We obtain $r_c =$ 0.131. In Section III D we compare the values obtained for r_c with those reported in the literature for a sedimentation experiment.

It is important to mention that K_i varies with the ionic strength of the solution. The values used here are the values for the ionic concentrations used in our experiments.

D. Interpretation of the DNA-drug complexes elasticity results

For low drug concentrations, drug intercalation in the DNA molecule increases the rigidity of the complex (see Figs. 2 and 4). This is consistent with the results of Vladescu *et al.* [21], which shows that EtBr stabilizes the DNA double-helix for low drug concentrations. They have performed melting experiments with various EtBr concentrations, from zero to 2.5 μM, showing that EtBr intercalation stabilizes the DNA double-helix in this concentration range. Therefore, we expect an increase of the persistence length of DNA-drug complexes in this low concentration range. Figure 4 shows this increase for EtBr, and Fig. 2 shows a similar result for daunomycin.

For high drug concentrations, *i. e.*, above the critical concentration (peak of Figs. 2 and 4), the persistence length of the complexes decays abruptly and remains practically constant.

It is well-known that intercalation unwinds the DNA double-helix [8]. Due to unwinding and above some drug critical concentration, the complexes can have a helix-coil transition, which can cause DNA denaturing as the DNA is stretched, decreasing the persistence length of DNA-drug complexes as seen in Figs. 2 and 4. The unwinding angle per intercalated EtBr drug molecule is approximately 1.7 times greater than that for daunomycin intercalation [8]. Therefore we expect that the transition occurs for EtBr at a lower drug concentration as compared with daunomycin, if the same DNA concentration is used. This is confirmed experimentally in our data of Figs. 2 and 4.

Sedimentation experiments performed with circular DNA as a function of daunomycin and ethidium bromide concentrations display a minimum in the sedimentation coefficient S_{20} at $r_c = 0.192$, for daunomycin and $r_c = 0.114$ for ethidium bromide [8]. The minimum in the sedimentation coefficient S_{20} is associated with a helix-coil transition, due to unwinding of the DNA double-helix by the intercalating drugs [8]. These numbers agree within 15 to 30% with the values of r_c determined from our DNA persistence length measurements. This indicates that the abrupt change of the DNA persistence length for both drugs might be also caused by a helix-coil transition due to the unwinding of the DNA double-helix as the drugs intercalate into it. The reasonable agreement between the critical ratios r_c obtained

from sedimentation experiments [8] and from our measurements of the persistence length transition provides an evidence that a helix-coil transition is probably what we are observing in our experiments.

In addition, it is known that EtBr (and also most intercalating drugs) exhibits multimodality at their interaction with DNA [22, 23]. The type of interaction varies with the drug concentration. The abrupt transition shown in Figs. 2 and 4 might as well be caused by different ways of drug binding to DNA.

IV. CONCLUSION

We have made systematic measurements of the entropic elasticity variation of a λ-DNA molecule when interacting with two drugs, daunomycin and ethidium bromide, as a function of their concentrations. Our results show that the persistence length of the DNA-drug complexes increases strongly as the drug concentration increases, for low concentrations. Above certain critical drug concentration the persistence length decreases abruptly and remains practically constant for high drug concentrations. This behavior is quite similar for both daunomycin and EtBr, as shown in Figs. 2 and 4. Our results suggests that the abrupt transition observed in the persistence length might be due to a helix-coil transition and denaturing of DNA-drug complexes above the critical concentration, resulting in a decrease of the persistence length.

V. ACKNOWLEDGEMENTS

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Figure Legends

Figure 1.

Force as a function of extension for a drug-free DNA molecule. By fitting this curve with Eq. 1, we determine the persistence length $A = 50 \pm 3$ nm and the contour length $L = 16.5 \pm 1$ μm.

Figure 2.

Persistence length A of DNA-daunomycin complexes as a function of drug concentration for fixed DNA concentration ($C_{bp} = 11$ μM). A initially increases with C_D until it reaches a maximum value (~ 280 nm) at the critical concentration $C_D^{critical} = 18.3$ μM. Then, the persistence length decays abruptly to around 75 nm and remains practically constant at this value even if we continue to increase the drug concentration.

Figure 3.

Force *versus* extension curves (normalized by the contour length) for two daunomycin concentrations. The data Brownian fluctuations are averaged out for better visualization. *Circles*: $C_D = 20.1$ μM (above the critical concentration) and $A \sim 61$ nm; *triangles*: $C_D = 18.3$ μM and $A \sim 263$ nm. Dashed lines are fittings using Eq. 1.

Figure 4.

Persistence length of DNA-EtBr complexes as a function of drug concentration for fixed DNA concentration ($C_{bp} = 11$ μM). Here, the transition occurs at a lower drug concentration. The maximum value measured for the persistence length of DNA-EtBr complexes is ~ 150 nm, at the critical concentration $C_E^{critical} = 3.1$ μM.

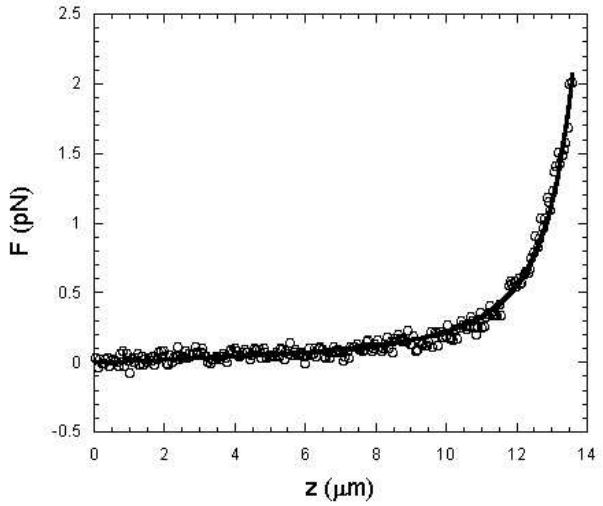


FIG. 1:

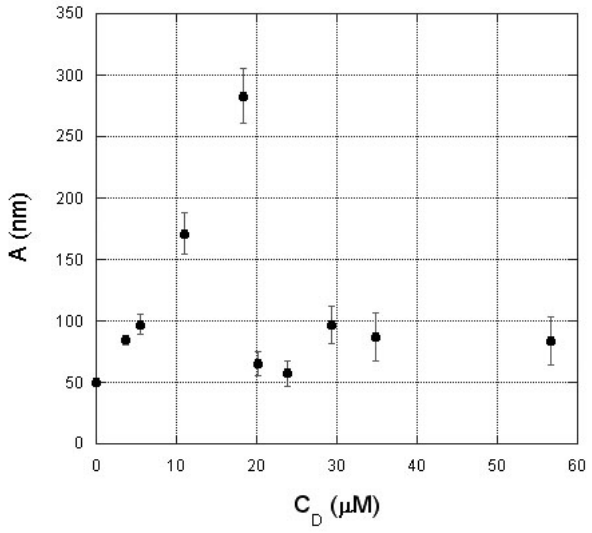
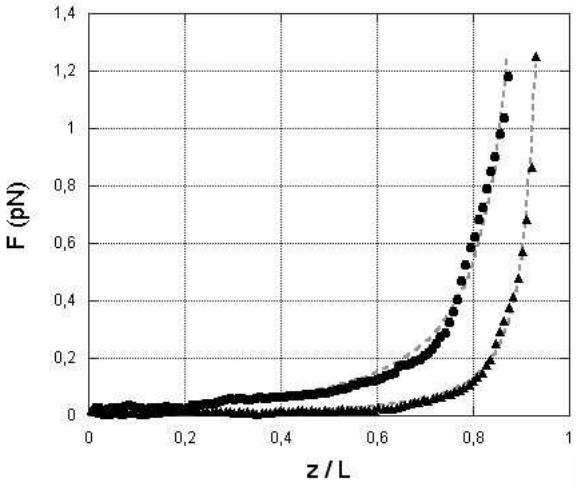


FIG. 2:



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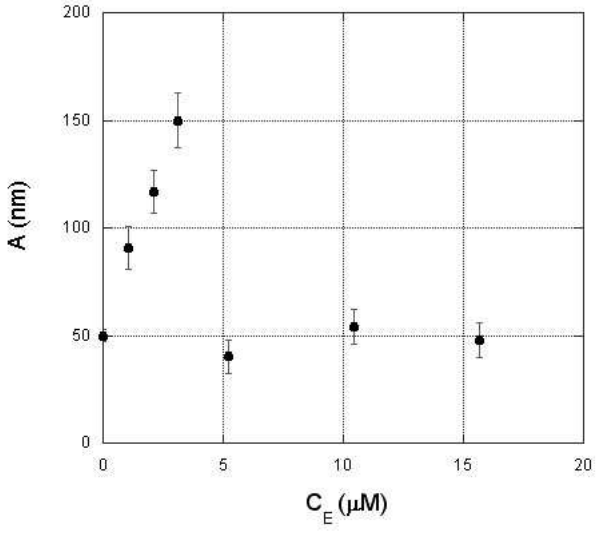

FIG. 4: